Indoor Moulds and Their Associations with Air Distribution Systems

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I. Introduction

A wide diversity of moulds may be isolated from indoor sites and, typically, somewhat fewer fungi can be shown to colonize indoor surfaces. We identify fungal colonization by the presence of vegetative growth represented by ramifying hyphae with particular attention for conidiogenesis or dividing yeast-like cells. The colonization may be temporal and microscopic or more extensive, obvious by sight and smell in buildings with moisture problems. Fungi within buildings without moisture problems exist mostly as dormant or transient forms. Many of these transients represent airborne or food-associated moulds from the outside environment, but indoor construction materials and furniture may also transport and harbor dormant endogenous moulds. Outside air typically bears a variety of mould propagules with the potential for colonizing damp indoor surfaces (Levetin *et al.*, 2002; Mishra *et al.*, 1992; Samson *et al.*, 1994).

We have found that cellulosic ceiling tiles and paper coatings of gypsum wallboard in the southeastern United States are especially

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vulnerable to colonization by species of *Acremonium, Alternaria, Aspergillus, Ascotricha, Chaetomium, Cladosporium, Penicillium,* and *Stachybotrys.* These genera and *Trichoderma* and *Syncephalastrum* may also grow on chronically dampened wood. Minor, and sometimes extensive, mould colonizations of indoor environments may be cryptic (e.g., air distribution systems, wall cavities) or even be observable but go unrecognized because the aesthetics of the indoor environment for the occupant had not been unsuitably altered.

Moulds, such as *Alternaria,* have long been recognized as allergens, but only about 5% of the population is estimated to show recognizable clinical illness, and within this group, the more severe hypersensitivity pneumonitis is rare (ACOEM, 2002). Fungal infections of humans, perhaps with the exception of aspergillosis, are associated rarely with moulds endogenous to indoor human habitats. In general, the classical fungal pathogens (e.g., *Coccidioides immitis*) that can cause grave diseases among "normal" or healthy individuals have an outdoor epidemiologic association with any indoor correlations (*Histoplasma* and *Cryptococcus*) related to deposits of bat or bird excrement. Aspergillosis occurs (or is recognized) predominantly among immunocompromised individuals within institutional healthcare settings. At least in the southeastern United States, our unpublished data and reports of others (e.g., Shelton *et al.*, 2002) indicate that species most commonly implicated in invasive aspergillosis (*Aspergillus fumigatus*, over 80%) occur indoors at an inverse incidence to the most common species, *A. versicolor* (over 90%), found indoors in hospitals and commercial buildings (Simmons *et al.*, 1997). We found *A. fumigatus* at high incidence and densities in woodchip ground cover near entrances and along walkways and play areas. We also found a relatively high incidence and high densities of *A. flavus* associated with filters in heating, ventilation, and air-conditioning (HVAC) systems in the southeastern United States. These latter two species are the most common etiological agents of invasive aspergillosis, followed at a distant third by *A. niger* (more often associated with otomycosis); *A. terreus* has been noted only recently at an increasing incidence (Sigler and Verweij, 2003). Infections of humans by the *A. versicolor-A. sydowii* complex are rare, and *Stachybotrys* spp. have not been proven to cause infections. Recovery of the various common moulds from indoor habitats may not necessarily correlate with a specific incidence of disease, including allergic fungal sinusitis, or even the diffuse complaints of the sick building syndrome (SBS) (CDC, 2000; Hospenthal *et al.*, 1998; Mishra *et al.*, 1992; Noble *et al.*, 1997; Shelton *et al.*, 2002; Singh and Singh, 1999).

The voluminous reports on serious adverse effects of "toxic" and "killer moulds" in the lay media have caused a dramatic increase in litigation and substantially increased costs of insurance and mould-related remediation (see Anonymous, 2003; Belkin, 2001).

A. Mycotoxins

Some mould species common in indoor habitats may produce secondary metabolites during their colonization of indoor substrates. These secondary metabolites may be toxigenic for other microorganisms. The mycotoxins from certain moulds (e.g., *Aspergillus flavus, Stachybotrys chartarum*) are known after ingestion to be toxigenic for animals, possibly including humans. Investigations have demonstrated that both toxigenic and non-toxigenic genotypes exist within these morphology-based species complexes and that toxin expression with growth on water-damaged building materials is affected also by environmental conditions (Andersson *et al.*, 1997; Andersen *et al.*, 2002; Cruse *et al.*, 2002; Nieminen *et al.*, 2002; Nikulin *et al.*, 1994; Peterson *et al.*, 2002; Ren *et al.*, 1998, 1999). Reports associating toxigenic moulds, particularly *S. chartarum*, with pulmonary hemorrhage in infants (Dearborn *et al.*, 1999) and neurological effects with adults (Johanning *et al.*, 1996) have raised recognition and controversy about potential health effects of indoor mould. Whether the toxins are produced during colonization of the indoor habitat in sufficient concentrations to produce hemologic or neurologic illnesses recognizable in humans via an inhalation route is unproven (ACOEM, 2002; CDC, 2000; Kuhn and Ghannoun, 2003; Page and Trout, 2001). The obfuscation of the health effects of indoor mould growth, in part, is related to the concomitant presence of the requisite damp conditions, dust mites, other allergens such as cockroaches, or overall low quality of the air (e.g., high CO_2) at complaint sites. In general, these overall conditions can be associated with an increased incidence of allergic responses and the severity of asthma attacks (NAS, 2000). A synopsis of the mycologic status of indoor mould investigations has been provided by Levetin *et al.* (2002).

II. Problem Areas with Air Distribution Systems

Our studies of indoor moulds have focused on colonization of surfaces, particularly in association with HVAC systems. Morey and Shattuck (1989) reviewed the major HVAC parameters that may result in adverse air quality for building inhabitants as follows:

Systems-Design Related: Outdoor air intakes are incorrectly placed so that they uptake exhausts or microbial-laden aerosols from plumbing vents, cooling towers, loading areas, etc.

Construction Related: Faults in the building envelope or plumbing leaks permit moisture to dampen susceptible substrates. Finishing materials such as wallboard are exposed to excessive moisture from joint compounds or fresh concrete in areas with restricted airflow. In these instances, the air distribution system may be engineered properly but its operation may facilitate the dissemination of fungi to susceptible substrata throughout the building.

Building-Operation Related: The operation of the air-conditioning system is cycled improperly so as to save energy.

Building-Maintenance Related: Air filters are not inspected or replaced as needed, and routine cleaning of cooling coils and condensation pans (particularly their drainage systems) is insufficient. Microbial populations in cooling towers are not controlled. Routine cleaning and rapid and proper response to moisture intrusions (catastrophic or chronic) are lacking.

Building-Renovation Related: A negative indoor pressure is present because local exhaust systems have been added without balancing intake supply. The negative pressure results in excessive intake of non-filtered and non-cooled air via portals aside from the designed air-conditioning system. Renovation of interior walls has obstructed internal air distribution.

Occupant Related: Human occupancy, or excessive electrical components such as computers, increase ambient temperatures beyond the capacity of the HVAC system.

All of the categories described by Morey and Shattuck (1989) indirectly or directly affect dew points within the building structure and thus microbial proliferation. Envelope leaks, chronic plumbing leaks, catastrophic floods, and particularly operation of intake fans at speeds excessive for the cooling capacity of the system were major factors leading to indoor mould colonization in our studies in the southeastern United States.

A. Air Samples

Periodic extensive sampling over several days that compares indoor and outdoor densities of airborne fungi may provide valuable information as to a potential source of fungi in the indoor environment. Differences between the species isolated may be as significant as differences in densities (Samson *et al.*, 1994). Air-sampling data alone, particularly

on a limited basis, however, can provide misleading information on the possible relationship of indoor fungi with the health status of the building's inhabitants. There is general agreement that numbers of fungi in indoor air, particularly for buildings that are not subject to complaints of poor air quality, are usually lower than the outdoor air. A large recent survey reported that the median indoor sample contained about 80 CFU/m^3, and outdoor samples showed median densities near 500 CFU/m^3 (Shelton *et al.*, 2002). These authors noted that no statistically significant association was observed between any common fungal type and reported health complaints (Shelton *et al.*, 2002). Robertson (1997) reported that 87% of 934 indoor air samples yielded <300 cfu/m^3, whereas only 33% of 182 outdoor samples were below this number, while 519 samples exceeded 500 cfu/m^3. Because no single enrichment medium is suitable for the growth of all fungi and because of differential growth rates and antagonisms between various common indoor fungi—and because systems for rapid fungal enumerations, identifications, and differentiations between colonizing fungi and transient dormant types from indoor reservoirs are not available—routine limited air sampling is impractical and provides minimal useful data. For these reasons we have made extensive use in our studies of clear adhesive-tape sampling of surfaces and "bulk" sampling (actual samples may be unobtrusive in size and be as small as several centimeters in each dimension) with incubation in moisture chambers or purge and trap vessels (Fig. 1A–C). The single-stage Andersen air sampler used in most of our studies is shown in Fig. 1D.

III. Colonization of Filters

A. Hospitals

Simmons and Crow (1995) and Simmons *et al.* (1997, 1997a) found that periodic colonization by moulds occurred on cotton-based filter media exposed to humid air. The colonization was not restricted to cotton filter media, however, or to fiber media with a heavy bioburden of organic debris; colonization of polyester fibers and even the colonization of HEPA microglass filters was observed (Fig. 2). Some unused clean-appearing filters were colonized microscopically and sometimes visually within 15 days. The cardboard support frames were the first to provide visual evidence of fungal growth, followed shortly thereafter by cellulosic filter media and usually, last, by polyester media. In several instances, however, with two-layered polypropylene-cellulosic bag filters, the polypropylene outer layer was colonized first (*Cladosporium*

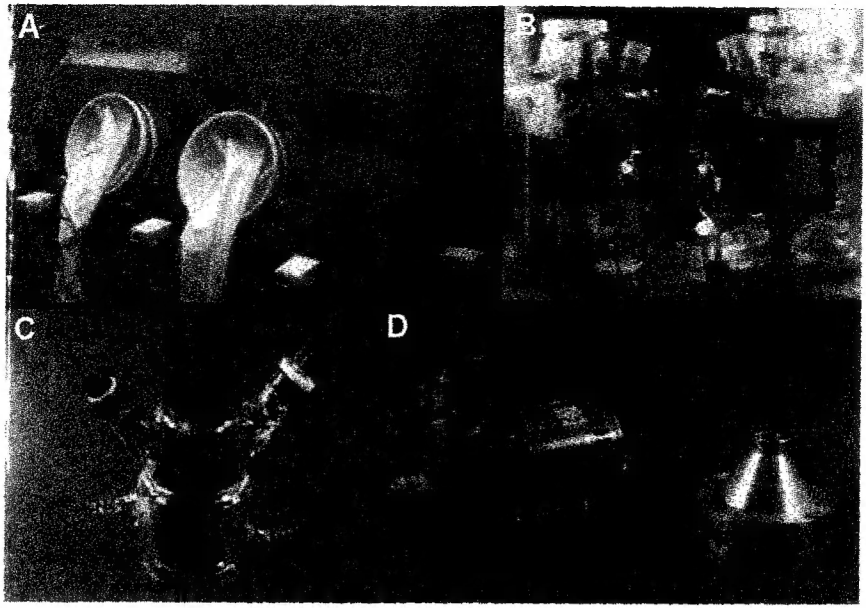

FIG. 1. Moisture chambers and air sampling apparatus. (A) Glove box with heater, air-circulating fan, and soil and water reservoirs; (B) vessels with and without soil reservoirs and various salt solutions for control of RH. Sections of various new and used materials were suspended over the reservoirs and examined periodically for development of microbial colonization. Chambers for certain experiments were initially sterile; (C) Purge and trap vessel for collection of VOCs; (D) Single-stage Andersen air sampler (Grasby Andersen, Atlanta, GA).

spp. in our studies), and fungal penetration into the inner preserved cellulosic layer was negligible. This may have been a result of preferential adsorption of volatile organics (hydrocarbon substrates) to the polypropylene. Our studies have indicated also that the binder for the filter media (ethylene vinyl chloride, which is more resistant to colonization than polyvinyl acetate) may be an additional influence on the rate and species of fungal colonization. In general, the spectra of species colonizing the filter frames were more diverse than those found colonizing the filter media. Additionally, species we more often associated with outdoor air (e.g., *Alternaria*, *Epicoccum*) colonized the frames but not the filter media. Often the dominant species that colonized filters in our studies were not the same as those that were dominant colonizers of downstream in-duct insulation (Price *et al.*, 1994; Simmons and Crow, 1995; Simmons *et al.*, 1997).

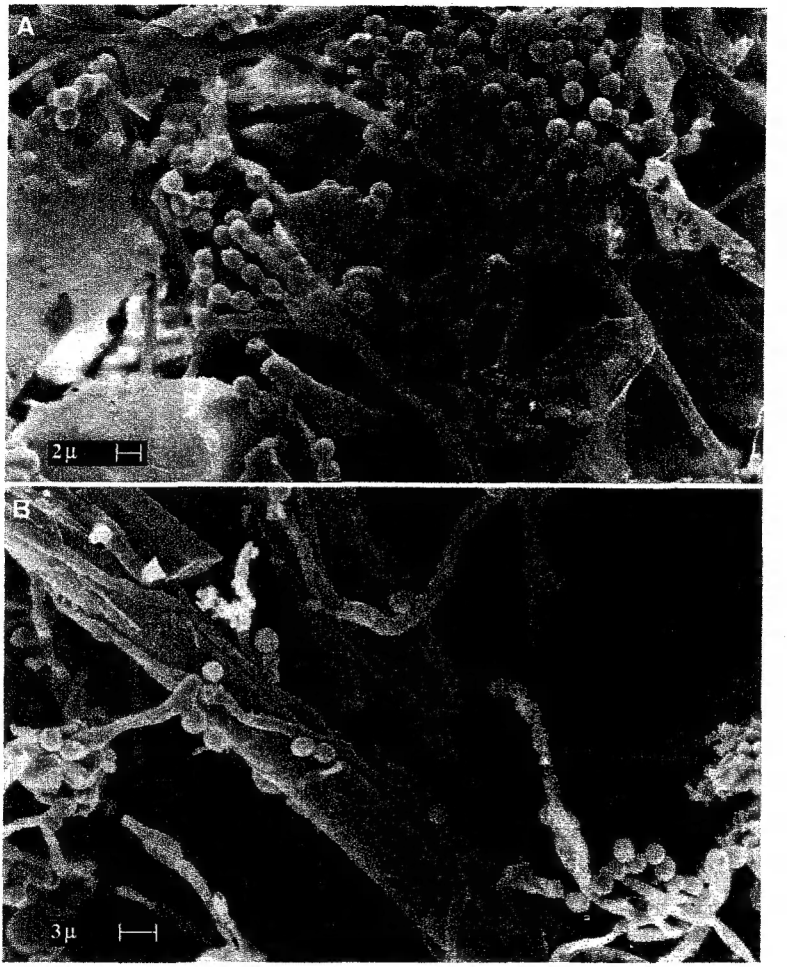

FIG. 2. Fungal colonization of a HEPA filter. (A) SEM of upstream side of the filter with micro colony of *Penicillium* sp.; (B) Downstream side of the filter with hyphae appressed to the microglass fibers and atypical (reduced) penicillus with conidia (examined immediately after removal from the air duct).

Our studies (unpublished) have included examination of filters from over 60 buildings (mainly healthcare facilities) and have included a comparison of filters treated with a phosphated-amine preservative (Intersept®, registered with US EPA for use in air-conditioning systems) versus non-treated filters. The filters were transferred to the laboratory and examined microscopically. Sections were placed in moisture

chambers. On used air filters, colonization was observed mostly on the filter media (usually cotton or polyester fibers). Entire filters with frames were not available from all sites. For filter samples that included the entire filter with beverage board (clay-coated, non-corrugated, reinforced cellulose) frame and filter media, the beverage board was often preferentially colonized (Table I). Approximately 58% of the untreated filters yielded fungi, versus 5% for the Intersept-treated. Among untreated filters examined, 20% were colonized on receipt, and 10.5% of these were from hospitals. Among the phosphated-amine treated filters, 13.4% were colonized on receipt, all of which were from hospitals. After moisture chamber exposure, 73% of the used untreated filters became colonized within 2 weeks, versus none of the used phosphated-amine treated filters.

In a separate set of experiments, 12 Intersept-treated filters were placed in a hospital HVAC system and monitored for fungal colonization over a 9-month period. Hospital engineers had reported moisture control problems in this system (Fig. 3A, B). Filters were constructed of solid pleated polyester cotton (80/20) media supported with beverage board frames and were positioned as secondary bank prefilters downstream of the heat exchange coils, drain pan, and system fan. During the cooling cycle, a cool mist saturated the filter frames and media. Untreated sections of the beverage board filter frame were colonized with *Alternaria, Aspergillus, Chaetomium, Cladosporium*, and *Penicillium* following 6 weeks in a moisture-laden hospital HVAC system with excessively low operating temperatures of 17–20°C. Exterior sections of filter frames coated with clear acrylic coating fortified with Intersept were free of fungal colonization; however, interior sections where the air filter media was attached to the frame were colonized with the above-cited fungi (Fig. 3C, D). Probable areas of antagonism between adjacent fungal colonies are suggested in Fig. 3E.

IV. Colonization of In-Duct Insulation

In several instances, extensive air sampling throughout the buildings did not reveal evidence of the presence of fungi growing within the HVAC systems (Ahearn *et al.*, 1992, 1996). Activation of the units after short periods of shutdown did not result in recoveries of fungi from the air at the outlets that were beyond the upper range of colony-forming units (about 25 cfu/m^3) in the ambient air of the building. Only when the unit was inactivated for weekend periods with sampling during the first hour after activation did the numbers of recoverable fungi significantly exceed (>300 cfu/m^3) ambient air numbers. Further

TABLE I

INCIDENCE OF FUNGAL COLONIZATION ON NON-PRESERVED AND PRESERVATIVE-TREATED FILTERS*

Year filter examined	Untreated, colonized upon receipt	Untreated, colonized post-moisture chamber	Untreated, no colonization	Intersept-treated colonized upon receipt	Intersept-treated colonized post-moisture chamber	Intersept-treated, no colonization
1995	8	29	4	1	0	11
1996	1	9	1	2	0	3
1997	5	13	1	2	0	11
1998	1	3	0	0	0	6
1999	1	6	0	1	0	3
2000	1	2	0	1	0	11
Total from hospitals	9	35	2	7	0	30
Total						
n = 137	17†	62†	6	7	0†	45†

*Used, non-preserved and preservative-treated (media only) air filters were collected from HVAC systems following varying periods of use. The filters were placed in clean plastic bags and brought to the laboratory for immediate microscopic examination. Sections of filters, 6 cm^2, were placed into initially sterile moisture chambers (IL wide-mouth polycarbonate jars with a plastic cup pedestal [bottom removed] and containing 200 ml of distilled water [>90% RH]) (see Price and Ahearn, 1999). The samples were incubated for 72–96 h and examined with light microscopy for evidence of fungal growth.

†Values are statistically significant with an unpaired t test ($P < 0.05$).

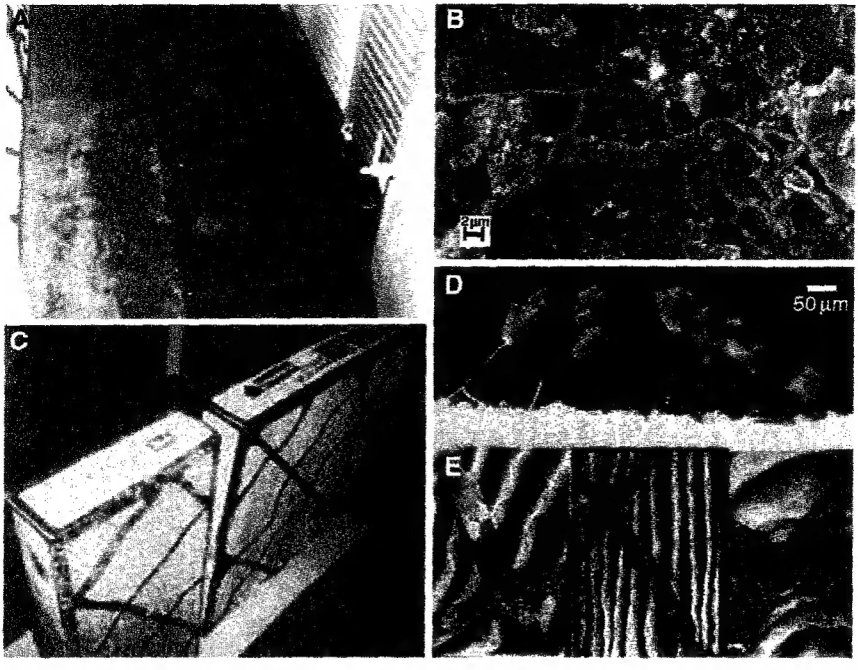

FIG. 3. (A) Improperly engineered drain pan filled with condensate; small squares in water are bars of sanitizing agent; (B) Hyphae colonizing surface of sanitizing bars; (C) Filters, non-preserved (left) covered with mould and preservative-treated (right) after three weeks in filter bank; (D) Well-developed conidiophores of *Aspergillus flavus* on non-preserved filter; (E) Various moulds on non-preserved filters with clear zones suggesting antagonisms.

examinations showed that the acrylic coating of in-duct fiberglass insulation was colonized by species of *Penicillium* and *Cladosporium*, with apparent successional periods of conidiogenesis, which varied with relative humidity. The time frames for colonization of various insulation sections placed in the ducts varied with the brand and even the placement of the fiberglass. Species of *Cladosporium*, *Penicillium*, and *A. versicolor* adhered tightly to the acrylic surfaces and were not always obvious from visual observations. Relative humidities of the ambient air were <85%. In general, conidiogenesis by *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* spp. could be detected on "tape mount"-microscopic examinations and specific staining of various sections of the fiberglass insulation (Fig. 4A, B). Detection of the in-duct colonizations was difficult at several sites, because access ports were

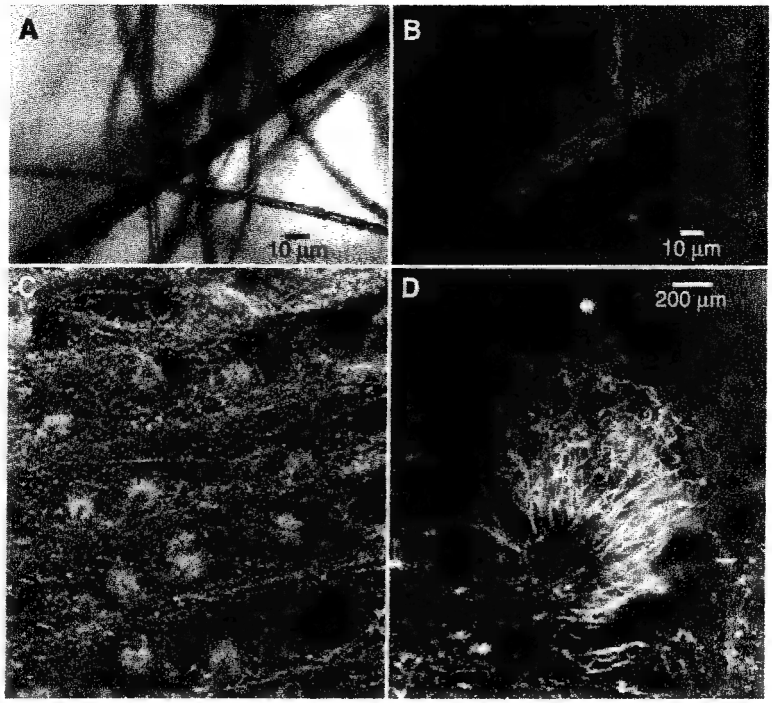

FIG. 4. Fungal colonization of fiberglass insulation. (A) Glass filaments; (B) Same field stained with calcofluor showing thin hyphal elements tightly appressed to glass filaments; (C) Surface of acrylic coating of in-duct insulation, the coating was supported by a fiber mesh with each section of the mesh supporting growth of *Chaetomium* sp.; (D) Enlargement of one of the mesh sections showing mature perithecium of the *Chaetomium* sp.

not always available when initial air sampling was performed (Ahearn *et al.*, 1996, 1997). We frequently isolated *A. versicolor* from used and new (less often) in-duct fiberglass insulation. In the presence of relative humidities from about 70% to 85% (dependent on the brand of insulation), colonization by the species permeated the insulation matrix (see Ezeonu *et al.*, 1994, 1995; Price *et al.*, 1994). Development of microscopic colonization of several types of unused in-duct insulation at low humidities occurred by the above fungi over a 30- to 90-day period at temperatures of 15° to 20°C both *in situ* and in moisture chambers. Bulk samples of one type of fiberglass insulation from three geographically separate buildings (Georgia, Louisiana, Texas) on transfer to moisture chambers also demonstrated profuse production of perithecia and ascospores by *Chaetomium* spp. (Fig. 4C, D).

A. Metal Surfaces

The metal ductwork itself and the supply vents can also become colonized, mostly on painted surfaces, by species of *Cladosporium* (Ahearn *et al.*, 1991). Since this study, unpainted metal ducts also have been found to be colonized by *Cladosporium, Rhinocladiella,* and occasionally *Acremonium.* Volatile organics apparently adsorb not only to fiberglass insulaton but also to the metal. Over time the a_w at

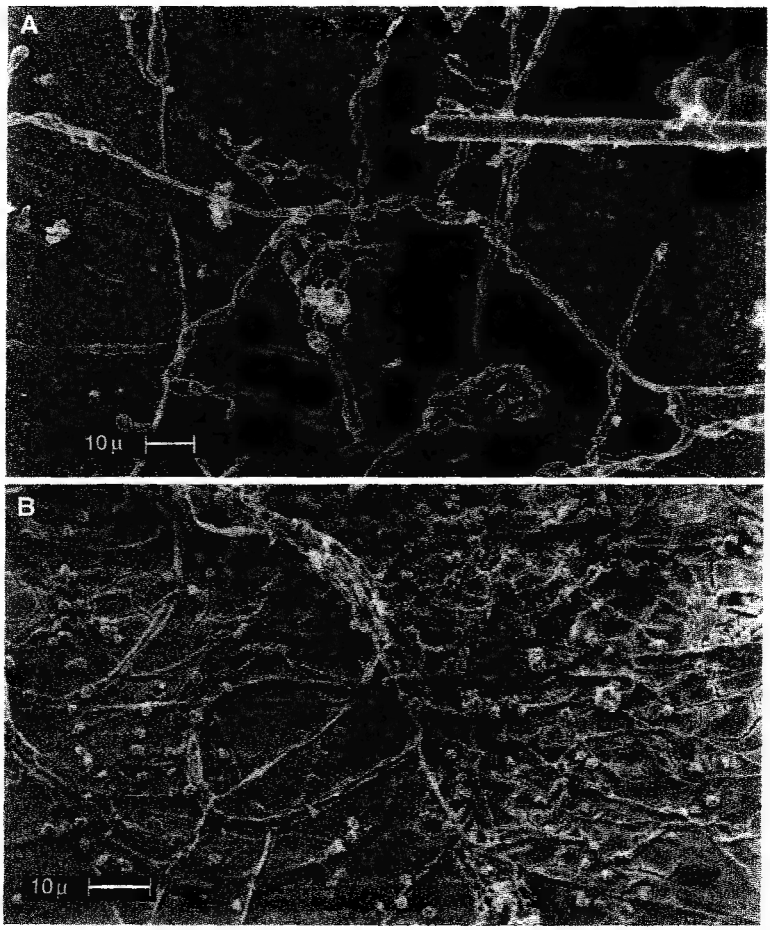

Fig. 5. Fungal colonization of bare metal surface of air duct; (A) *Cladosporium* sp.; (B) *Acremonium* sp. Growth and conidiogenesis apparently supported by adsorbed organics from air stream.

the organic-air interphase often supports growth of *C. cladosporioides* on the metal surfaces. When the metal surface is a site of chronic condensation, *Acremonium* can occur (Fig. 5).

B. Volatile Organic Carbons

There is a general consensus in the literature that moulds, particularly *A. versicolor*, one of the more common indoor fungi, have the capacity to contribute to the indoor levels of irritant organic volatiles (Bayer and Crow, 1993; Bjurman and Kristensson, 1992; Ezeonu *et al.*, 1994; Larsen and Frisvad, 1994; Pasanen *et al.*, 1997). Often olfactory senses are the only indication of microbial colonizations within buildings. The spectrum of volatiles produced varies with environmental conditions such as temperature, relative humidity, substratum, and the presence of other organisms (Wilkins *et al.*, 2000). We found that ethanol, benzene, and the odiferous ethyl hexanol were among volatiles released from fiberglass insulation colonized by *A. versicolor* and *Acremonium obclavatum* (Bayer and Crow, 1993; Ezeonu *et al.*, 1994). Pasanen *et al.* (1997) found also that ethyl hexanol (2-ethyl-1-hexanol) 1-octen-len-3-ol, 2-pentanone, 2-hexanone, 2-heptanone, 3-octanone, and 2-methyl furan were produced following inoculum of *A. versicolor*.

Most of the common indoor fungi—particularly strains of *Aspergillus, Penicillium*, and *Chaetomium*—perhaps with the exception of *Stachybotrys chartarum* and *Cladosporium cladosporioides*, readily produce olfactory volatiles with growth on a variety of substrates under damp conditions. In some of our test systems, the numbers and concentrations of volatiles that were released from various substrata decreased with development of the mixed microbial biofilms (Rose *et al.*, 2002).

Our preliminary studies suggest that volatiles may play a role in growth and conidiogenesis of certain fungi within buildings. We found that vapors of ethyl acetate, ethyl hexanol, and propanol retarded germination of several common moulds on Sabouraud's dextrose agar, whereas pentane and hexane had no effect or stimulated germination (Table II). The time for germination in the presence of acetone varied with the species. The stimulative effect for pentane and hexane was suggested by the trend for more rapid development of the germ tube versus the water control. When plates of Sabouraud's agar were inoculated with selected species and incubated at 22°C to 24°C in the presence of selected volatiles, microscopically (100×) and visually detectable growth, generally equivalent to the control, was evident by 24 h in the presence of most of the compounds inhibitory for germination in water

TABLE II

EFFECTS OF VOLATILE ORGANIC COMPOUNDS (VOC) ON THE GERMINATION OF FUNGI

	Species of *Aspergillus* and *Penicillium**							
VOC	AV	AN	PV	PCO	PS	PC	PG	PGR
Acetone	0†	88	17	93	100	0	0	0
Ethanol	0	0	0	54	0	24	ND	0
Ethyl acetate	0	0	0	0	0	0	0	0
Ethyl hexanol	0	0	0	0	0	0	0	0
Hexane	83	100	91	97	100	100	100	100
Octane	ND	ND	ND	92	100	ND	ND	ND
Pentane	100	100	100	95	100	100	67	100
Propanol	0	0	0	0	0	0	0	0
DH_2O	93	100	91	100	100	100	95	100

*AV = *A. versicolor*, AN = *A. niger*, PV = *P. viridicatum*, PCO = *P. corylophilum*, PS = *P. sclerotiorum*, PC = *P. chrysogenum*, PG = *P. glabrum*, PGR = *P. griseofulvum*, ND = Not determined.

†Average % germination: (n = 10). Conidia were harvested from 5–7 days growth (25°C) on Sabouraud's dextrose agar, washed and suspended in 0.9% saline (~10^6 conidia/ml); organic compounds (100 μl/10 mm diameter cellulose disks); 0.1 ml of suspension of conidia was spread evenly on surface of Sabouraud's agar (15 ml in 100 mm Petri dish) and 1.0 cm^2 sections of agar were transferred to prescored microscope slides that were placed in a glass Petri dish (<1.0% germination at 0 time) containing the VOC disk; the dish was sealed and after 18 h at 25°C the number of germinated and total conidia were determined in each of five alternate sections and the average percent germination determined; each test system for each VOC were maintained in duplicate-separate-sealed chambers. Data are from a representative experiment of three similar tests with varied but overlapping spectra of species.

agar. Conidiogenesis, however, appeared to be stimulated for isolates of *A. versicolor* and *A. fumigatus* in the presence of hexane.

V. Carpets and Wallboard

Carpets in the hallways of two of the buildings described above were wet-cleaned over weekends and permitted to air dry with the air-conditioning system turned off. Water from the carpets was absorbed into the gypsum wallboard under the plastic base cove. This resulted in the cryptic growth of *Aspergillus* and *Penicillium* under the cove on the wallboard with conidia release at the cove borders. There was detectable odor in the hallways, and *Penicillium* spp. were recoverable in cultures made from the carpets. The carpets (synthetic fibers) were infrequently found to be colonized, but often they served as reservoirs for the conidia that were seeded periodically from the colonizations

within the air ducts (Price and Ahearn, unpublished data). A relatively new set of carpets was replaced in one building, but the new carpets rapidly became culture positive (Ahearn *et al.*, 1996, 1997). The presumption on the basis of positive Rodac plates (obtained by an outside consultant) was that the carpets were supporting active growth. The fungal colonization in the air ducts and behind the base coves was cryptic. Upon removal of the in-duct insulation for about 9 m beyond the air handling units, and constant running of the units over the weekends, particularly when the carpets were cleaned, complaints of musty odors ceased (Ahearn *et al.*, 1996, 1997). New carpets in other buildings were found on occasion to have had high densities of moulds prior to their installation (unpublished data).

Incorporation of antimicrobial preservatives in the backing of commercial carpet tile has been found to inhibit microbial growth, staining, and odors (Fig. 6A) (Price *et al.*, 1991). For example, 8-year-old carpet tiles with the phosphated-amine preservative incorporated at the backing were removed from a neonatal intensive care unit (NICU) in a southwestern US teaching hospital and examined for fungal colonization and retention of inhibitory activity. Initial examination of the soiled carpet via direct microscopy showed that the carpet was not colonized but harbored viable conidia and other particulates. Enrichment culture of small discs (4 mm diameter) of the soiled carpet tile yielded mainly bacteria (*Bacillus* spp.) with colony development distal to the actual carpet sample and no colony development on the carpet disc sample. These results suggested that the inhibitory effect of the preservative was still present. The soiled carpet was sprayed with a stabilized hydrogen peroxide (3%) based carpet sanitizer, allowed to stand 15 minutes, and vacuum extracted (Fig. 6B). The NICU carpet tiles, cleaned with stabilized hydrogen peroxide (3%) based carpet sanitizer, were then challenged with *Aspergillus niger* and *Serratia marcescens* (10^5 cfu/ml). The 8-year-old carpet once cleaned, resisted overgrowth by both *Aspergillus niger* and *Serratia marcescens* in the agar overlay challenge (Fig. 6C).

In contrast to the synthetic carpets discussed above, natural fiber carpets, particularly with jute backing and including wool and cotton, are more readily subject to colonization by many of the common indoor fungi. We have found species of *Ascotricha* and *Trichoderma* colonizing wool fibers of carpets and have found *Aspergillus* species colonizing the backing of non-preserved synthetic carpets. In most instances, sources of periodic or chronic moisture intrusion were readily associated with these colonizations (Fig. 7). Carpet pads, especially those laid directly on unsealed concrete, are particularly subject

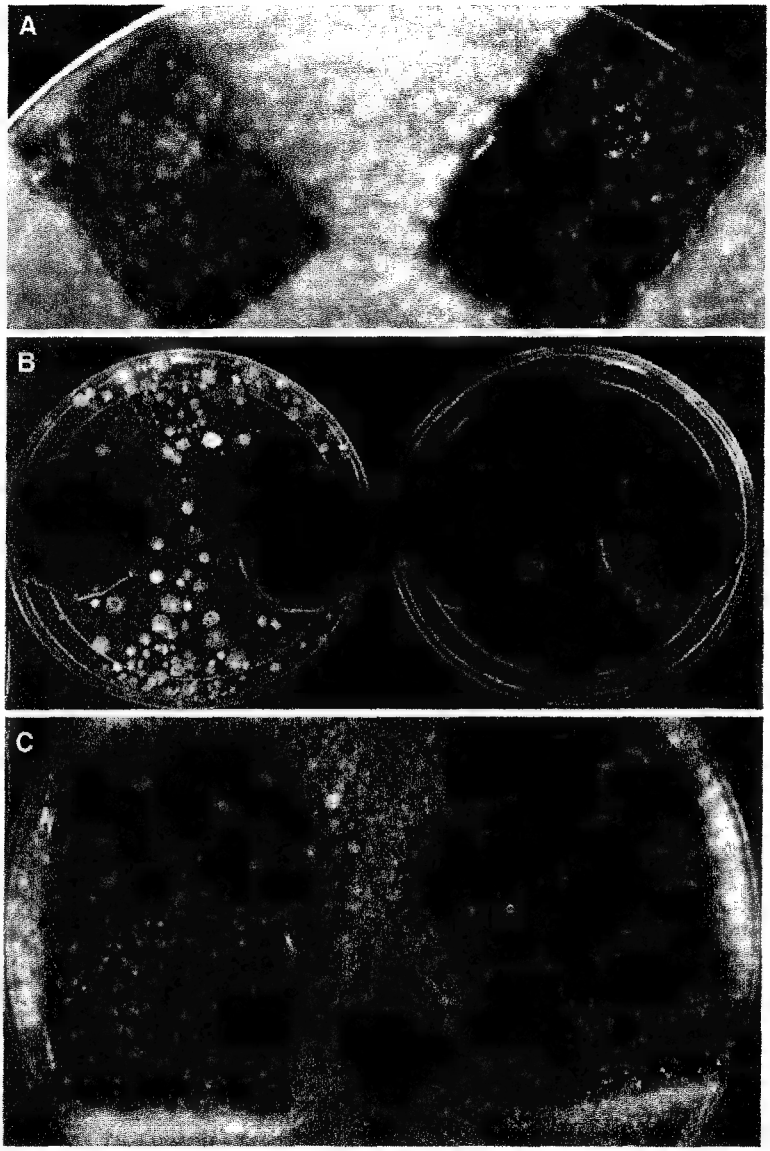

FIG. 6. (A) Enrichment culture of non-preserved (left) and preserved (right) sections of synthetic carpet tile challenged with *Aspergillus niger*. (B) Recovery of dormant or transient microorganisms from 8-year-old preserved carpet tile (left); minimal recovery from same carpet after cleaning with stabilized hydrogen peroxide (right). (C) Inhibition of *A. niger* in enrichment agar by preservative in cleaned 8-year-old used carpet tile. Backing of carpet (lower sections with fibers shaved off) resists fungal overgrowth (see Price *et al.*, 1991, for details of methods).

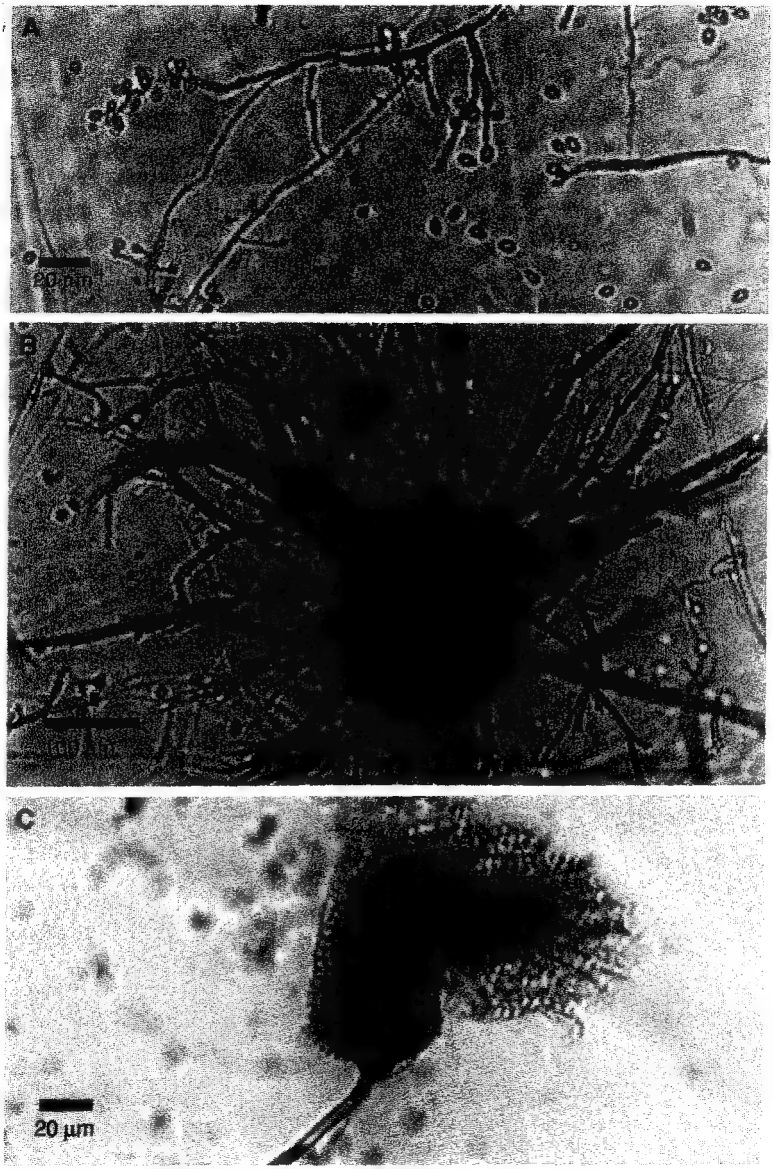

FIG. 7. (A) Anamorph (*Dicyma ampullifera*) conidiophores with conidia from adhesive tape of wool carpet; (B) Perithecium of *Ascotricha* sp. (anamorph *D. ampullifera*) with sympodial branching filaments; (C) Conidiophores and conidia of *Aspergillus terreus* developing from bitumen layer of synthetic non-preserved carpet (three weeks in moisture chamber).

to contamination by both bacteria and fungi. In a number of cases, adhesive to hold the padding on the concrete or sub flooring was subject to colonization.

A. *Stachybotrys chartarum*

Stachybotrys chartarum is not an uncommon mould in water-damaged buildings and is particularly associated with gypsum wallboard and cellulosic ceiling tiles (Fig. 8). On damp wallboard, under plastic base coves and vinyl wall coverings, black areas with dense masses of conidia were found on microscopic examination of tape mounts. These colonized areas were often interspersed with sections colonized by

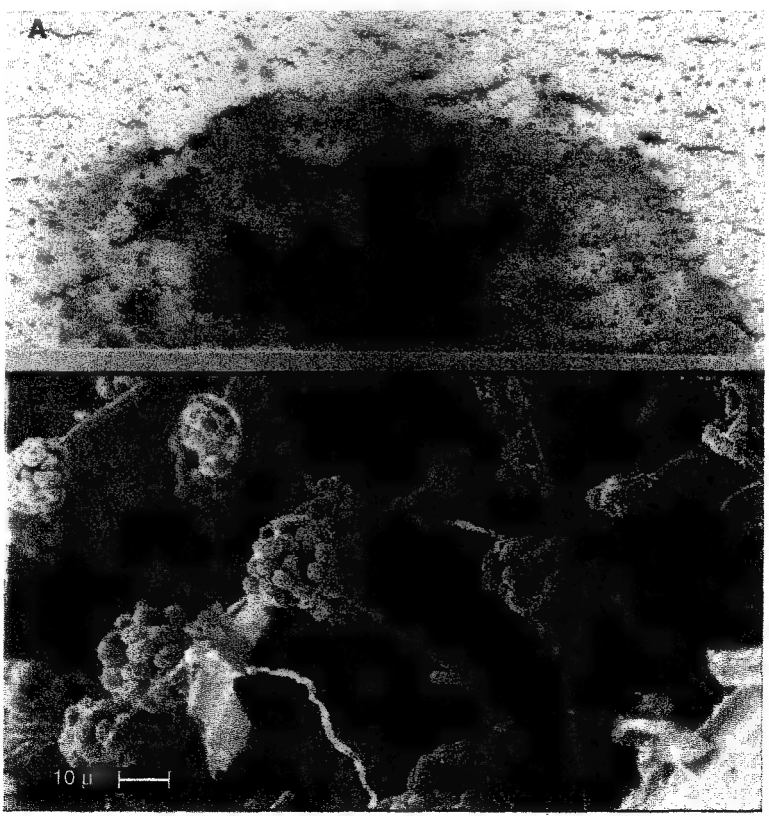

FIG. 8. (A) Stained ceiling tile supporting growth of *Stachybotrys chartarum*; (B) SEM of conidiophores and conidia of *S. chartarum* on cellulose ceiling tile.

asexual stages of *Ascotricha* and *Chaetomium*, with perithecia of the latter not uncommon. Nevertheless, swab cultures of these areas on malt extract agar were prone to be overgrown by *Aspergillus* and *Penicillium* spp. and air samples from the immediate areas, with minor exception, failed to provide for isolation of *S. chartarum*, even when a variety of media were employed. Andersen and Nissen (2000) found that (of 22 media examined) malt extract agar supported the growth of isolates of *S. chartarum*, but 2 out of 8 isolates did not sporulate. All tested isolates of *S. chartarum* and *C. globosum* also grew on potato-sucrose agar and V8 juice agar, but two did not sporulate. None of the 22 media were suitable for the detection of all the targeted fungi. We have observed similar variances in germination, growth, and conidiogenesis by these fungi on the cellulosic surfaces of wallboard (Price and Ahearn, 1999). Fine vegetative hyphae (not detectable with the unaided eye) may permeate the cellulose coating of wallboard with about 25% water content. After three to four weeks incubation at 95% + relative humidity (about 30% water content), sectors may blossom with pigmented conidiophores bearing conidia. This occurs more rapidly in the *Ascotricha* > *Chaetomium* > *Stachybotrys*. How generally this observation applies is problematic, because we have not differentiated the metabolic types within the *S. chartarum* complex (Andersen *et al.*, 2002; Cruse *et al.*, 2002).

Of 17 buildings in the Georgia-Florida area in which we studied colonizations of *S. chartarum*, only on three occasions could we associate the species with the air distribution system. In one building, the paper-glue layer under the foil coating of fiberglass insulation that jacketed the HVAC ductwork was extensively colonized. Similarly, we found *S. chartarum* under foil coating of rigid fiberglass ductwork. These insulations, the fiberglass matrices of which yielded *A. versicolor*, had been reportedly exposed to moisture during construction (Price *et al.*, 1995). On another occasion, we isolated *S. chartarum* from a cotton rag that had been stuffed into a wall unit air conditioner to prevent vibration. About half the buildings colonized with *S. chartarum* were complaint sites (SBS) and for all but three, once the moisture and aesthetic problems had been alleviated, complaints ceased.

VI. Colonization of Automobile Air-Conditioning Systems

Mixed fungal and bacterial biofilms occur not uncommonly within automobile air-conditioning systems with chronic or periodic release of noxious odors (Kumar *et al.*, 1990; Rose *et al.*, 2000; Simmons *et al.*, 1997). Air-conditioned vehicles with entrapped condensate anywhere

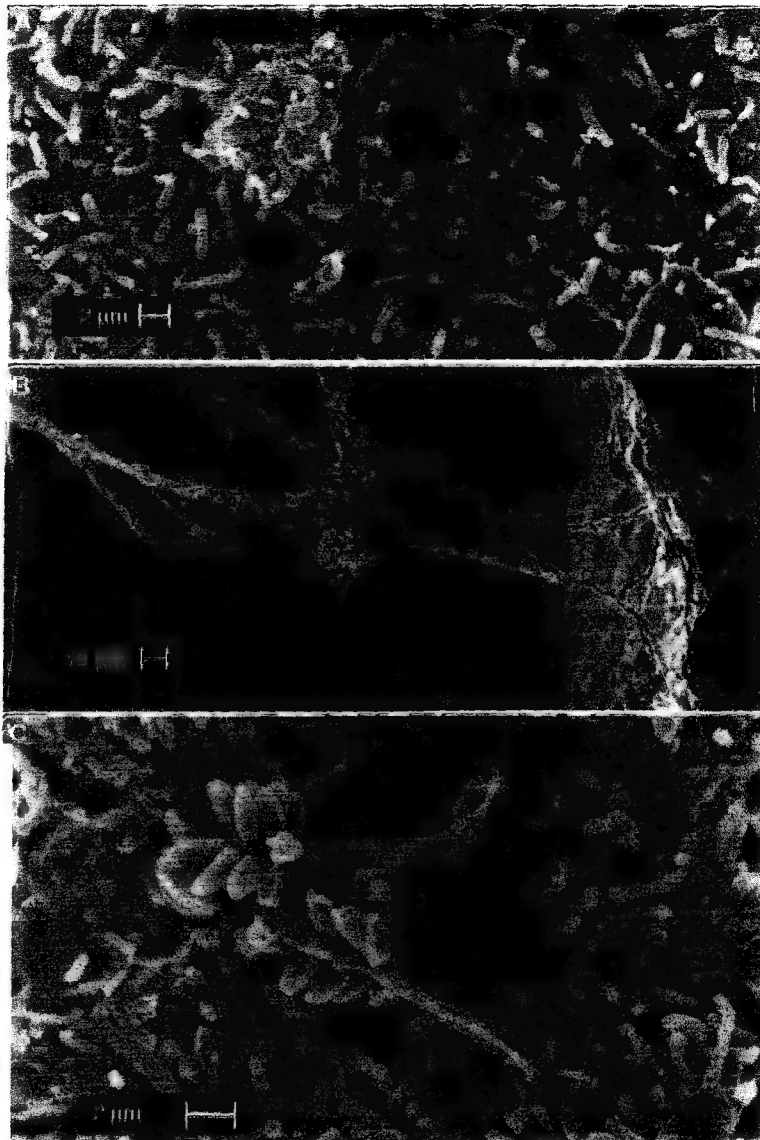

FIG. 9. (A) *Acanthamoeba* sp. cyst in a mixed bacterial biofilm on an evaporator-heat exchanger fin; (B) Hyphae traversing open celled foam insulation surrounding the evaporator core; (C) Mixed fungal (*Rhinocladiella* sp.)—bacterial biofilm on surface of a drain tray.

in the system are subject to microbial colonization. Multiple components—air filters, insulation foams, rubber gaskets, drain tubes, and even the aluminum evaporator fins—may support microbial biofilms (Fig. 9). These biofilms may develop after only short-term use of the vehicle. The actual production of odors and fungal conidia and their release into the passenger compartment of the automobile may be dependent on the chance initiators of the diverse biofilms that can be present. In certain instances, bacteria may predominate; in others, fungi. The occurrence and distribution of fungal species in automobiles may be affected by the presence of predatory *Acanthamoeba* spp. With some model automobiles, the problem may be alleviated when the automobile is parked level so that the condensation pan fully drains.

Running the AC-system blower while the automobile is parked for a few minutes before turning off the ignition also may aid in the evaporation of condensate. With certain ACs, the adoption of components noncompliant for microbial attachment and redesign of the condensate drainage system has been necessary. When the above approaches are not available or economically practical, treatments to retard microbial development in the systems may suffice (Drago *et al.*, 2002).

VII. Conclusions and Recommendations

Fungal colonization of indoor materials is not uncommon but is often cryptic, particularly when it occurs within the air distribution system and within walls. Extensive use of adhesive tape-sampling, combined with direct microscopic examination of surfaces and incubation of bulk samples at specific humidity ranges, has proven helpful in distinguishing colonizing from transient and dormant fungi.

Moisture control provides for indoor mould control; however, all modern habitats with air-conditioning systems (or available water) will have microenvironments that support mould growth. At some time in the life of a structure, catastrophic or chronic water intrusions will occur that could, if not remediated, support mould growth. Moulds, endogenous to the structure or entering on a daily basis, will be present to initiate the colonization. Halting the excess water intrusion and rapidly drying the site will most often be sufficient for preventing mould growth that could adversely impact the building's inhabitants. The relatively inexpensive operation of a portable dehumidifier in basement areas will frequently suffice for controlling musty odors in residences following discreet incidences of water intrusion. If visible mould colonization develops on gypsum wallboard or some other surfaces, but structural integrity has not been compromised, cleaning

with a sanitizing agent such as a 10% bleach solution could suffice (CDC, 2001). Such sanitation steps (which should include subsequent drying of the material) may also be followed by application of antifungal preservative coatings. If massive fungal growth is present and structural surfaces need to be replaced, a sanitation step for dry surfaces should be considered, with removal of materials while surfaces are still damp. Efficient respiratory protection that will exclude dust particles and standard protective clothing, gloves, safety glasses, etc., should be sufficient for normal (excluding hypersensitive or immunostressed) workers performing the remediation. If the moulds achieve ambient particle counts of species (such as aspergilli) within a restricted airflow and cfus common to counts found for composting areas (e.g., $\geq$10,000 cfu/m^3), more stringent recommendations might be advised (see EPA, 2001). In all instances, the air-conditioning vents to the affected sites are sealed (including return air) immediately prior to any remediation activities. It seems unreasonable to assume that surfaces, particularly nonporous types or even fabrics that have been colonized by moulds, cannot be safely sanitized without major expense. In fact, some of the more notorious moulds (i.e., *S. chartarum*) appear relatively susceptible to sanitizers (Price and Ahearn, 1999).

Certain fungi are allergens, and fungal exposures may elicit low incidences of hypersensitivity pneumonitis or allergic fungal sinusitis, particularly in the immunocompromised individual. Although these maladies have been more frequently recognized in the past several decades, they have not as yet shown high incidences of definitive associations with SBS or fungal colonization with a specific indoor habitat (Noble *et al.*, 1997).

Our experiences indicate that in many instances, indoor materials can be sanitized to an acceptable level and that the use of preserved materials combined with good maintenance can significantly reduce the extent of indoor mould colonization. In most of our published studies, we have examined a phosphated-amine preservative of low water solubility and low toxicity. Foarde and Menetrez (2002) also examined this preservative and found it effective in sealants for retarding fungal development in fiberglass duct liner and on galvanized steel.

There is no question that musty indoor odors produced by mould growth are unpleasant and can significantly reduce the quality of indoor air for all inhabitants. For certain allergic individuals, mould growth may make particular materials such as clothing, furnishings, or even the habitat unacceptable. The number of individuals whose health are significantly affected by indoor moulds is unknown, but

undoubtedly fewer people are affected than intimated in some investigations and in the recent popular press.

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